Time-Related Mapping of Quantitative Trait Loci Underlying Tiller Number in Rice

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ABSTRACT

Using time-related phenotypic data, methods of composite interval mapping and multiple-trait composite interval mapping based on least squares were applied to map quantitative trait loci (QTL) underlying the development of tiller number in rice. A recombinant inbred population and a corresponding saturated molecular marker linkage map were constructed for the study. Tiller number was recorded every 4 or 5 days for a total of seven times starting at 20 days after sowing. Five QTL were detected on chromosomes *1*, *3*, and *5*. These QTL explained more than half of the genetic variance at the final observation. All the QTL displayed an S-shaped expression curve. Three QTL reached their highest expression rates during active tillering stage, while the other two QTL achieved this either before or after the active tillering stage.

THE advent of molecular marker technology has introduced a new era to the science of quantitative genetics. Since late 1980s, molecular markers have been used extensively to map quantitative trait loci (QTL). However, most of the QTL-mapping studies have been limited to analyzing the performance of a trait (the word "trait" used in this article will always refer to the quantitative trait) observed at a fixed time or stage (usually the end) of ontogenesis. Such a QTL-mapping strategy may be called time-fixed mapping (TFM), which can only estimate the effects of individual QTL accumulated from the beginning of ontogenesis to the time of observation. According to developmental genetics, the development of a trait must result from differential activities of many related QTL. This means that different QTL may have different expression dynamics during the trait development, even though they may have the same final effects. Therefore, to understand the genetic functions of QTL thoroughly, we should know, not only their effects at a given time or stage, but also their expression dynamics. For this reason, time-related (phenotypic) data obtained by successive observations throughout the trait development should be used for QTL analysis. Such a strategy of QTL mapping may be called time-related mapping (TRM). Apart from revealing the expression dynamics of individual QTL, TRM can also increase the statistical power for QTL detection because repeated observations on the same individuals over different ages are a form of replication, so more genetic information is used (Knapp and Bridges 1990; Verhaegen *et al.* 1997).

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The developmental genetics of quantitative traits (or developmental quantitative genetics) has been studied for several decades on the basis of the methodology of conventional quantitative genetics (Kheiralla and Whittington 1962; Peat and Whittington 1965; Wu 1987; Xu and Shen 1991; Zhu 1995). In recent years, molecular markers have been applied to map QTL and to estimate their effects in different developmental stages or periods (Bradshaw and Stettler 1995; Sondur *et al.* 1995; Plomion *et al.* 1996; Price and Tomos 1997; Verhaegen *et al.* 1997). These studies have provided some evidence of differential activities of QTL during ontogenesis. There are generally two approaches to TRM. One is to analyze trait values observed at sequential times (Bradshaw and Stettler 1995; Plomion *et al.* 1996; Price and Tomos 1997; Verhaegen *et al.* 1997), from which the accumulated effect of a QTL, from the beginning of ontogenesis to each observation time, can be estimated. We call this effect cumulant analysis (ECA; Wu *et al.* 1997). The other approach is to analyze trait value increments observed at sequential time intervals (Bradshaw and Stettler 1995; Sondur *et al.* 1995; Plomion *et al.* 1996; Verhaegen *et al.* 1997), from which the incremental effect of a QTL at each time interval can be estimated. We call this effect increment analysis (EIA; Wu *et al.* 1997). The characteristics of ECA and EIA have been investigated in detail by Wu *et al.* (1997).

For both ECA and EIA, phenotypic data from different times or time intervals can be analyzed either separately or jointly. As joint analysis could take the information of genetic correlation between different times or time intervals into account for QTL mapping (Jiang and Zeng 1995), it might have some advantages in comparison with separate analyses. Until now, however, almost all

the studies of time-related QTL mapping have adopted only the separate analyses approach. An exception is the study conducted by Verhaegen *et al.* (1997). They applied ANOVA to jointly analyze data from different developmental stages, but ANOVA cannot estimate the effects of QTL.

Tiller number is an important agronomic character in rice and is easily measured throughout its development. Hence, tiller number could serve as a suitable model trait for the study of TRM of QTL. In this article, we report a study of TRM of QTL underlying the development of tiller number in rice by using the method of composite interval mapping (CIM; Zeng 1994) for separate ECA (SECA) and separate EIA (SEIA) and the methods of multiple-trait CIM (MCIM; Jiang and Zeng 1995) for joint ECA (JECA) and joint EIA (JEIA).

MATERIALS AND METHODS

Experiment and map construction: A population consisting of 131 recombinant inbred (*RI*, F_7) lines was constructed by a single-seed descent from a cross between two indica rice varieties, H359 and Acc8558. A field experiment was carried out at Fujian Agricultural University in China in 1996. Seeds of the *RI* lines and their parents were pregerminated on May 26 and sown in germination plates on May 31. Seedlings were transplanted into the field on June 15, with five seedlings per line planted in a row. Fifteen seedlings of each parent were planted in three separate rows that had been arranged randomly among the *RI* lines. To reduce competition among individuals, a wide distance of 33 cm between plants and between rows was adopted. The tiller number of each plant was investigated every 4 or 5 days from June 20 to July 18, *i.e.*, at 20, 24, 29, 33, 38, 43, and 48 days after sowing (denoted as t_1, t_2, t_3, t_4, t_5, t_6, and t_7, respectively). The investigation ended at t_7 because some young tillers began to die after that time.

Using the computer software MapMaker (Lander *et al.* 1987), a dense molecular marker linkage map with 225 marker loci (including 147 RFLPs and 78 AFLPs) covering a length of 1435.8 cM was constructed on the basis of the *RI* population. Markers in the map were approximately evenly distributed, but there were still some clusters where markers were very closely linked (distance < 1 cM) and nearly cosegregated. Obviously, only one marker in each cluster was needed for QTL mapping; all others would be redundant and, therefore, could be omitted. After removal of these redundant markers, a total of 199 markers were then used for the QTL analysis. Details of the map are to be published in another paper.

QTL analysis: Both CIM and MCIM were used for separate and joint ECA and EIA. For an *RI* popluation, if epistatic effects are neglected, the model of MCIM can be written as (note that CIM can be taken as a special case of MCIM when only one trait is involved)

$$y_{jk} = b_{0k} + b_k^* x_j^* + \sum_l^m b_{lk} x_{jl} + e_{jk} \quad (j = 1, \ldots, n;\ k = 1, \ldots, t), \tag{1}$$

where y_{jk} is the phenotypic value of individual j for trait (or time or time interval) k; b_{0k} is the mean of the model for trait k; b_k^* is the additive effect of the putative QTL on trait k; x_j^* is an indicator variable of the putative QTL's genotypes, taking values of 1 for Q_1Q_1 and -1 for Q_2Q_2, with probabilities depending on the genotypes of flanking markers (Wu *et al.* 1996); b_{lk} is the partial regression coefficient of y_{jk} on marker l; x_{jl} indicates the genotypes of marker (cofactor) l, taking values of 1 for M_1M_1 and -1 for M_2M_2; e_{jk} is the random error for trait k in individual j; and n, m, and t are the numbers of individuals, markers selected as cofactors, and traits to be analyzed, respectively. In matrix notation, Equation 1 can be further expressed concisely as

$$\mathbf{Y} = \mathbf{X}\,\mathbf{B} + \mathbf{E}, \tag{2}$$

where $\mathbf{Y}$ is an $n \times t$ matrix of y_{jk}; $\mathbf{X}$ is an $n \times (m + 2)$ matrix of 1, x_j^* and x_{jl}; $\mathbf{B}$ is an $(m + 2) \times t$ matrix of b_{0k}, b_k^*, and b_{lk}; and $\mathbf{E}$ is an $n \times t$ matrix of e_{jk}.

Equation 1 or 2 can be fitted with the method of maximum likelihood via the ECM algorithm as proposed by Jiang and Zeng (1995), but its computation is complicated and time consuming. To simplify the computation, for the case of one-trait analysis, it has been shown that the method of least squares is also applicable to interval mapping (Haley and Knott 1992) and CIM (Wu *et al.* 1996) as long as the indicator variable x^* takes its expected value, determined by the genotypes of flanking markers, and the results are very close to those obtained by maximum likelihood. This conclusion could reasonably be extended to the case of multiple-trait analysis. In fact, it is apparent that Equation 1 or 2 is a standard, multivariate, multiple-regression model when the putative QTLs position is given. Hence, a standard least-squares procedure (*cf.* Press 1972) can be applied. The least-squares estimate of $\mathbf{B}$ is

$$\hat{\mathbf{B}} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{Y}, \tag{3}$$

the matrix of residual sum of squares is

$$\begin{aligned}\hat{\mathbf{E}}'\hat{\mathbf{E}} &= (\mathbf{Y} - \mathbf{X}\hat{\mathbf{B}})'(\mathbf{Y} - \mathbf{X}\hat{\mathbf{B}}) = \mathbf{Y}'\mathbf{Y} - \hat{\mathbf{B}}'\mathbf{X}'\mathbf{X}\hat{\mathbf{B}} \\ &= \mathbf{Y}'\mathbf{Y} - \hat{\mathbf{B}}'\mathbf{X}'\mathbf{Y},\end{aligned} \tag{4}$$

and the unbiased estimator of covariance matrix is

$$\hat{\sum} = \frac{1}{n - m - 2}\,\hat{\mathbf{E}}'\hat{\mathbf{E}}. \tag{5}$$

This algorithm can be computed easily.

The null hypothesis here is H_0: $\mathbf{b}^* = (b_1^*, \ldots, b_t^*)' = \mathbf{0}$ (reduced model, *i.e.*, the putative QTL does not exist), and the alternative hypothesis is H_1: $\mathbf{b}^* = (b_1^*, \ldots, b_t^*)' \neq \mathbf{0}$ (full model, *i.e.*, the putative QTL exists). Following the Box approximation approach (*cf.* Press 1972), we have a likelihood ratio (LR) statistic of

$$\mathrm{LR} = -(n - m - 1/2\ t - 2)\ln\frac{|\hat{\sum}_{\text{full}}|}{|\hat{\sum}_{\text{reduced}}|}, \tag{6}$$

where the subscripts full and reduced indicate that the covariance matrices are estimated from the full and reduced models, respectively. Under H_0, the LR in Equation 6 approximately follows a chi-square distribution with t d.f. when the putative QTL's position is given. Because the test is performed in the whole genome, however, the distribution of the maximum LR (or LOD $\approx$ 0.217 LR) score over the whole genome is very complicated. Therefore, it is difficult to determine theoretically a suitable significance threshold for the test, and, thus, empirical methods are usually required. In the present study, for each CIM or MCIM analysis, a permutation test (Churchill and Doerge 1994) with 1000 replicates was conducted to estimate the significance threshold.

Before MCIM was conducted, a multivariate stepwise regression (MSR) procedure was performed to select informative markers as cofactors. The regression model used was similar to Equation 1 or Equation 2, except that all the independent variables were markers. A significance test was performed on

each marker according to Equation 6. A significance level of 0.05 was used for the stepwise regression. Considering that our *RI* population was not large and there were more markers than *RI* lines, cofactor selection (stepwise regression) was conducted on each chromosome separately, and, when a marker interval was tested in CIM or MCIM, only the informative markers on the same chromosome were used in the model as cofactors so as to have a large degree of freedom. Also, a window was set for the marker interval being tested so that statistical power would not be reduced dramatically. Only the markers outside the window could be used as cofactors. Five window sizes (*i.e.*, 0, 5, 10, 15, and 30 cM on each side of the marker interval being tested) were tried to examine the influence of window size on QTL mapping.

In addition to being estimated by MCIM, effects of QTL at different times and time intervals were also estimated by MSR on the basis of a multiple-QTL model consisting of all the detected QTL as independent variables. With the estimates of each QTL's effect cumulant at each time [denoted as $a_i(t_k)$ for QTL i at t_k] and effect increment at each time interval [denoted as $a_i(t_{k+1} - t_k)$ for QTL i at t_k–t_{k+1}] obtained by MCIM or MSR, the effect growth curve (or expression curve) and effect growth rate curve (or expression rate curve) of each QTL were plotted. The effect growth rate (or expression rate) was calculated by $a_i(t_{k+1} - t_k)/(t_{k+1} - t_k)$. It could also be calculated by $[a_i(t_{k+1}) - a_i(t_k)]/(t_{k+1} - t_k)$. Theoretically, these two methods are equivalent (Wu *et al.* 1997).

The heritability of a QTL (*i.e.*, the proportion of phenotypic variance explained by the QTL) at a time (or similarly, at a time interval) was estimated by $a_i^2(t_k)/V_P$, and the heritability of all detected QTL was estimated by $[\Sigma_i a_i^2(t_k) + \Sigma_{i\neq j}(1 - 2R_{ij})a_i(t_k)a_j(t_k)]/V_P$ (Zeng 1993), where $R_{ij} = 2r_{ij}/(1 + 2r_{ij})$, r_{ij} is the recombination frequency between QTL i and j, and V_P is the phenotypic variance of the population.

RESULTS

Development and variation of the trait: Both the *RI* lines and their parents showed an S-shaped growth curve for tiller number. The fastest growth time interval (or the active tillering stage, ATS) was t_4–t_5. The tiller number, however, continued to increase until the final observation. Variation of tiller number increased as the trait developed. The difference in the final tiller number between the parents was 21.07 (H359 > Acc8558). In the *RI* population, the variation range, phenotypic variance, genetic variance (estimated by ANOVA), and heritability of tiller number at the final observation were 13.6–55.4, 55.01, 46.87, and 85.20%, respectively. This indicates that there was great genetic variation in tiller number among the *RI* lines, and the heritability was high. Hence, the population was ideal for QTL mapping. In addition, the mean values of the *RI* population were always approximately at the midparent point throughout the experiment. This implied that the additive model would be suitable for analyzing the data.

Locations of QTL: Empirical significance thresholds of LOD statistic at the genome-wise significance level of 0.05 (denoted as $LOD_{0.05}$) for JECA and JEIA were 6.32 and 6.01, respectively, when window size was zero. There were merely slight changes of $LOD_{0.05}$ under other window sizes. All SECA and SEIA (including all window sizes) also had very similar $LOD_{0.05}$, ranging from 2.93 to 3.12. Hence, window size had little influence on $LOD_{0.05}$.

TABLE 1

Putative QTL detected by TRM

	*tn*1[a]		*tn*3a[a]		*tn*3b[a]		*tn*3c[a]		*tn*5[a]	
	LOD[b]	Pos.[c]	LOD[b]	Pos.[c]	LOD[b]	Pos.[c]	LOD[b]	Pos.[c]	LOD[b]	Pos.[c]
SECA										
t_1	2.55	52	2.74	28	2.23	34	3.14*	0	1.64	68
t_2	2.47	52	2.95	28	3.93*	35	2.51	0	3.04*	69
t_3	2.05	53	3.58*	28	4.50*	35	0.69	0	4.94*	68
t_4	2.11	53	4.47*	28	4.96*	34	0.85	0	4.42*	67
t_5	1.77	53	5.14*	28	6.34*	35	0.53	0	5.63*	69
t_6	3.28*	53	5.61*	28	7.09*	35	0.30	0	5.42*	68
t_7	4.11*	55	4.94*	28	6.65*	35	0.29	0	4.34*	72
SEIA										
t_1–t_2	0.60	53	0.93	28	3.20*	36	0.25	0	3.07*	73
t_2–t_3	1.37	53	3.07*	28	3.78*	34	0.05	0	5.06*	67
t_3–t_4	1.71	53	4.31*	28	4.28*	34	0.80	0	3.07*	67
t_4–t_5	0.99	53	4.31*	28	6.46*	37	0.14	0	5.46*	71
t_5–t_6	4.44*	57	1.88	28	2.89	34	—	—	1.27	65
t_6–t_7	1.54	60	0.53	26	0.56	40	0.10	7	0.48	56
JECA	6.44*	52	3.42	28	4.55	35	1.97	0	6.43*	69
JEIA	5.94	56	3.97	28	6.44*	35	0.57	0	6.38*	69

[a] QTL's name *tn* is the abbreviation of tiller number; the numeral indicates the chromosome on which the QTL is located, and the lowercase letter indicates alphabetically the order of QTL on the same chromosome.
[b] Peak value. The asterisk represents significance at the 5% genome-wise level.
[c] Position in centimorgans corresponding to the peak of LOD score.

When there was no window (*i.e.*, window size = 0 cM), a total of five QTL were detected on three chromosomes, *i.e.*, chromosomes *1*, *3*, and *5* (Table 1). No extra QTL were detected when the window size increased. On the contrary, the QTL *tn*3a could not be detected when the window size was >5 cM. Hence, it seemed that no window was necessary for this study, so only the results of window-free analyses are listed in Table 1. SECA detected all five QTL, SEIA detected four of them, and JECA and JEIA both detected two QTL, respectively. This result implies that separate analysis is more powerful than joint analysis for QTL detection.

Both SECA and SEIA obtained very similar estimates of each QTL's position at different times and time intervals, although LOD peaks of the QTL were not statistically significant at some times and time intervals (Table 1). This indicates that the separate analyses across developmental stages were consistent. In addition, estimates of each QTL's position obtained by both JECA and JEIA, though not all statistically significant, were almost exactly the same (except for *tn*1), and they were also very close to the estimates obtained by SECA and SEIA (Table 1). Hence, it seemed that all these analyses were consistent. The relative positions of the QTL are shown in Figure 1 according to the estimates obtained by the joint analyses (note that for *tn*1, using the average of JECA and JEIA, *i.e.*, (52 + 56)/2 = 54).

Expression dynamics of QTL: Expression curves and expression rate curves of each QTL estimated by MCIM, *i.e.*, JECA and JEIA, and MSR are shown in Figure 2. Except for *tn*3a, all QTL showed positive effects. This means that alleles of these QTL from parent H359 acted to increase the character (tiller number). According to Figure 2, the curves obtained by MCIM and MSR were quite similar in shape, but the effects (both cumulants and increments) of the QTL estimated by MCIM were always greater than those estimated by MSR. Moreover, it was found that, according to the estimates obtained by MCIM, genetic variances explained by the detected QTL at different times and time intervals all exceeded the phenotypic variances. This means that MCIM has overestimated the effects of the QTL. The reason might be that the influence of unlinked QTL was not statistically controlled in the MCIM analyses in this study. As the population was not large, unlinked QTL might contribute to the estimate of the effect of the putative QTL being tested because of sampling errors. MSR solved this problem to some extent because all detected QTL were included in the model. In addition, it is noted that the QTL *tn*3c was not statistically significant in the MSR analysis. In fact, *tn*3c was detected only at the first observation by SECA (Table 1). This indicates that either the effect of *tn*3c across the trait development was very small (Figure 2, a and b) or that the detected QTL was actually a false positive.

All the QTL showed an S-shaped expression curve similar to the phenotypic growth curve (Figure 2). It is seen that the QTL had discriminative expression dynamics and greatly different final effects. The QTL *tn*3a, *tn*3b, and *tn*5 displayed the highest expression rate (or the most active expression) at time interval t_4–t_5, coinciding with the ATS; *tn*3c, if it existed, showed the highest expression rate at t_3–t_4, before the ATS; and *tn*1 showed the highest expression rate at t_5–t_6, after the ATS. The expression rates of *tn*3a and *tn*5 became close to zero after t_6 (Figure 2d), implying that they had basically ceased expression after that time. *tn*3c expression stopped even earlier. *tn*1, however, maintained a relatively high expression rate at the final time interval, with a potential of continued expression until the end of the tillering stage.

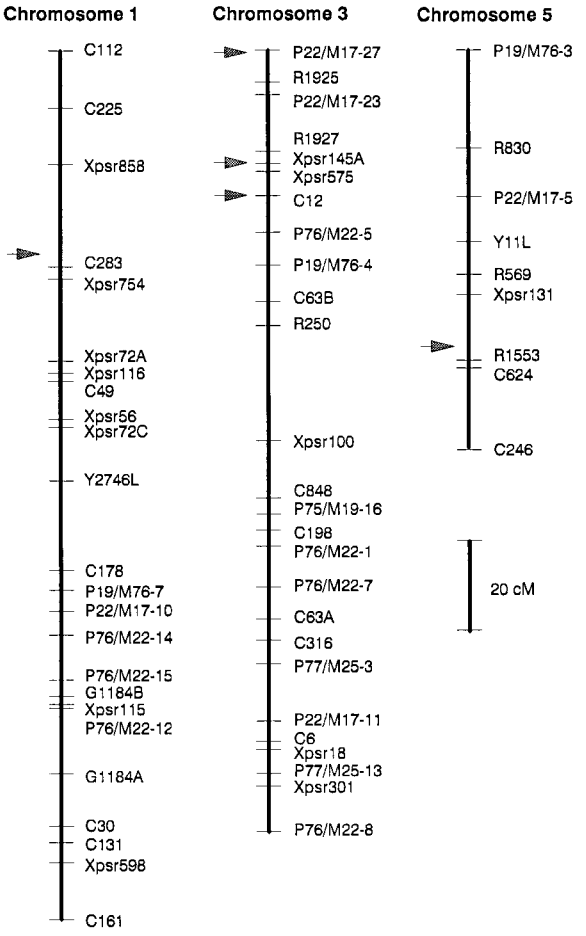

Figure 1.—Relative positions (indicated by arrows) of QTL underlying tiller number in rice. P−/M−, AFLP markers; Xps−, RFLP markers revealed by wheat probes; all others, RFLP markers revealed by rice probes. In chromosome *3*, the arrows from top to bottom indicate *tn*3c, *tn*3a, and *tn*3b, respectively.

The heritability of each QTL and that of all QTL at every time and time interval were estimated according to the results of MSR (Figure 3, a and b). The four significant QTL, *i.e.*, *tn*1, *tn*3a, *tn*3b, and *tn*5, displayed a heritability of 46.16% and explained 54.18% genetic variance in total at the final observation. This implies that there must be still many other QTL with minor effects explaining nearly half of the total genetic vari-

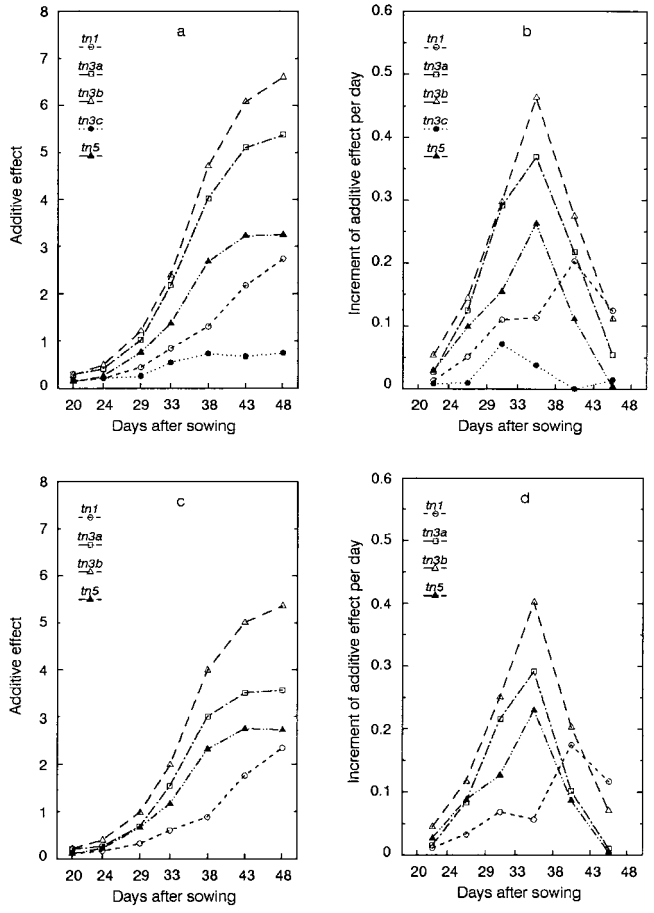

Figure 2.—Expression curves (a and c) and expression rate curves (b and d) of QTL underlying tiller number in rice. (a) Estimated by the JECA; (b) estimated by the JEIA; (c and d) estimated by MSR.

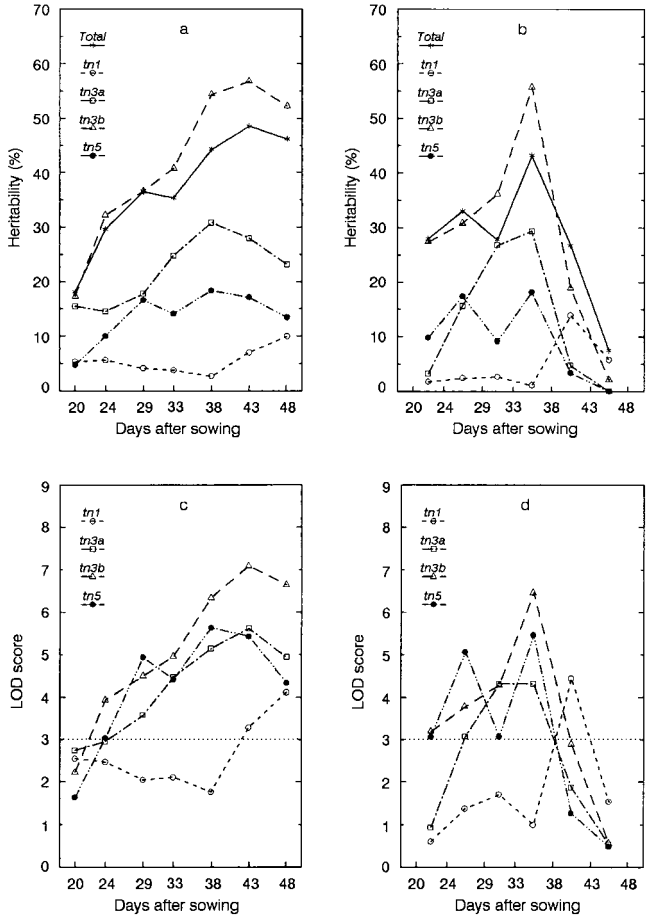

Figure 3.—Variation of heritabilities (a and b) and LOD scores (c and d) of QTL underlying tiller number in rice, as well as observation time (a and c) and time interval (b and d).

ance not detected in this study, probably because of the relatively small population size. It is interesting that the trend of variation of each QTL's LOD (peak) score across times (Figure 3a) and time intervals (Figure 3b) was quite similar to the variation trend of the QTL's heritability (Figure 3, c and d) rather than to the variation trend of the QTL's effect (Figure 2, c and d). This indicates that the probability of detecting a QTL is mainly determined by the relative contribution of the QTL to the phenotypic variation rather than by the absolute size of the QTL's effect. Therefore, at least in some cases, it could be improper to infer a QTL's expression status at a time interval in accordance with the result of SEIA because a QTL's being not statistically significant (because of small heritability) at a time interval does not necessarily mean that the QTL does not express (*i.e.*, its expression rate is zero) at that time interval (Wu *et al.* 1997).

DISCUSSION

TFM *vs.* TRM: It has been pointed out in the Introduction that TRM has two significant advantages over TFM: (i) it can reveal expression dynamics of QTL and (ii) it can increase statistical power for QTL detection. We have seen that the statistical power of detecting a QTL is largely determined by the QTL heritability, which varies with the trait development (Figure 3). This means that for each QTL, there must be a time (or times) and a time interval (or intervals) at which the QTL displays its maximum heritabilities and, consequently, maximum statistical power of being detected (Wu *et al.* 1997). TRM enables us to map QTL at their corresponding maximum statistical power times and time intervals; therefore, it can increase the statistical power for QTL mapping. On the contrary, with the strategy of TFM, only some of the QTL at most can be mapped at their corresponding maximum statistical power times (but not time intervals). In the present study, there was no observation time at which all the QTL could be detected (Table 1). Therefore, if QTL mapping had been carried out only at a fixed time (no matter what the time was), it would not have been able to detect as many QTL as the TRM did.

ECA *vs.* EIA: As cumulants and increments can be converted mutually, these two methods are, at least in theory, equivalent in the estimation of a QTL's effect. Their statistical power for QTL detection, however,

could be quite different. According to a simulation study for the case of separate analysis (Wu *et al.* 1997), ECA is generally suitable for mapping a QTL with a large final effect (*i.e.*, effect at the final observation time) or one that begins expression early although its final effect is small, while EIA is advantageous in mapping a QTL that starts expression late or that has a small final effect but a high expression rate that peaks within a narrow time interval during the period of trait development.

Joint *vs.* separate analysis: According to the results of the present study, it seems that SECA and SEIA based on CIM can detect more QTL than JECA and JEIA based on MCIM. This indicates that joint analysis based on MCIM is not necessarily statistically more powerful than separate analyses based on CIM, although in theory, the former always has a larger likelihood ratio (or LOD score) than the latter (Jiang and Zeng 1995). The advantage of joint analysis is that it can synthesize all the information from different times or time intervals to give a comprehensive estimate of each QTL's position, according to which a corresponding complete expression (or expression rate) curve of each QTL can be estimated. In practice, therefore, both separate and joint analyses should be conducted. In addition, joint analysis can be applied to the investigation of QTL expression dynamics of not only a single trait as in the present study but also multiple traits. This will allow us to study the developmental genetic basis of correlations among quantitative traits.

QTL mapping in small populations: Both CIM and MCIM require a large population (Zeng 1994; Jiang and Zeng 1995). In practice, however, population size usually cannot be very large. As in the present study, the population consisted of only 131 lines, but the map used contained 199 markers. In this case, selecting informative markers as cofactors becomes a problem. We once tried a two-step selection procedure. Markers on each chromosome were first screened separately with a relatively low significance level, and the preselected markers from different chromosomes were then screened jointly with a higher significance level. We found, however, that when different significance levels for the preselection were used, the markers that were selected finally could be quite different and, therefore, the putative QTL detected could also be quite different. Such inconsistency makes it very difficult or even impossible to determine which QTL detected are believable and, therefore, may seriously hurt the reliability of QTL mapping. In addition, integrating all the markers selected from the whole genome into the model could also greatly decrease the degrees of freedom for regression and, therefore, increase the error of parameter estimation. Hence, in this article, we have adopted a method of selecting cofactors and mapping QTL on each chromosome separately. The method could basically keep the desirable property of the interval test of CIM and MCIM and, at the same time, achieve larger degrees of freedom for a small population. A problem caused by this method is that the effect of the putative QTL being tested is generally overestimated because the possible contribution from unlinked QTL to the putative QTL resulting from sampling errors is not controlled. However, with the method of MSR based on a multiple-QTL model to estimate the effects of the detected QTL, the problem can be solved, at least to some extent. We think that this could be an appropriate approach for QTL mapping in small populations. In addition, setting a window for each marker interval being tested may also be required for a small population. In accordance with the result of this study, we suggest that a series of different window sizes should be tried in practical studies to identify the most appropriate one(s). There could be a possibility that different QTL or chromosomal regions need different window sizes.

Theoretical and practical meanings of TRM: The significant advantages of TRM as a new QTL mapping strategy have been demonstrated. However, it is necessary to stress that the importance of TRM is not limited to mapping. Because TRM can reveal the expression dynamics of QTL, it has actually moved the study of QTL mapping into an important research field—developmental quantitative genetics. Studies in this field will enable us to gain more and deeper insight into the genetic basis of quantitative traits, and they will also benefit plant and animal breeding.

The results obtained in the present study may provide useful information for rice breeding. An important goal of rice breeders is to create new varieties without nonproductive tillers because nonproductive tillers generally do not contribute to yield. As late developing tillers are usually nonproductive, they waste nutrition and energy. Therefore, the QTL that remain active until the late tillering stage (*e.g.*, *tn*1 in this study) could genetically cause nonproductive tillers. With the knowledge acquired in this study and the technology of marker-assisted selection, it might be possible in the near future to design and create ideal genotypes for new rice varieties with few nonproductive tillers.

While TRM has been clearly demonstrated here to be of value in studying the dynamics of tiller development, the technique will also be of considerable use in studying other characters. For example, at the present time, soluble stem carbohydrates are believed to be of value in enabling cereal plants to resist drought, as the carbohydrate reserves can be mobilized to move from stems into developing grain during times of water stress. TRM could help to elucidate the genetic control of the carbohydrate reserves over time in different tissues and, thus, aid breeders who aim to breed more drought-resistant plants. Another important use of TRM will be in studying complex pathogens, such as Fusarium of wheat, that can attack plants at the juvenile stage (foot rot) or the adult plant stage (head blight). It is believed that some genes for Fusarium resistance may act at cer-

tain stages during the plant development, while others act throughout the life cycle. Sequential disease testing combined with TRM should, thus, enable breeders to select genes that are active throughout the plant life cycle.

Finally, it is necessary to point out that the concept of QTL expression studied here is only at the phenotypic level. Revealing its relationship to the molecular or biochemical process would be a future task for developmental quantitative genetics. At the present time, care should be taken in deducing QTL expression dynamics at the molecular or biochemical level from the results obtained from studies at the phenotypic level.

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